Transient neonatal cyanosis associated with a new Hb F variant - Hb F Viseu

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Abstract

Neonatal cyanosis in healthy newborns can be associated either with methemoglobin due to cytochrome b5 reductase deficiency or to M-Hemoglobin, a group of hemoglobin variants resulting from mutations in the globin chain genes. We report the clinical case of a neonate with cyanosis and normal cardiac and respiratory function. At birth the hematological parameters were normal; however the methemoglobinemia was 16%. Spontaneously the cyanosis gradually decreased and by the fifth month of age the methemoglobin level was normal. An heterozygous Gγ globin gene (HBG2) missense mutation 87 C-A (Leu28Met) was identified. His father, with a past history of transfusion in the neonatal period, is heterozygous for the same mutation. This hemoglobin variant, not previously described, was called Hb-F-Viseu and is the sixth G gamma chain variant reported in association with neonatal cyanosis.

Key words: Neonatal cyanosis, M- Haemoglobin, methemoglobinemia

Introduction

Congenital cyanosis is associated with disorders occurring with deoxygenated hemoglobin, such as heart and airway malformations, hemoglobin variants with low oxygen affinity and methemoglobinemia due to cytochrome b5 reductase deficiency or M-hemoglobin variants. Methemoglobin (metHb) is the result of hemoglobin iron moiety oxidation from the ferrous to the ferric state, compromising oxygen exchange and oxygen supply to tissues thus causing cyanosis. Under normal circumstances multiple endogenous agents induce the production of metHb; however significant accumulation is prevented by the action of the enzyme NADH-metHb reductase (also known as cytochrome b5 reductase), keeping metHb levels below 1%. Newborns are more prone to acquired methemoglobinemia as fetal hemoglobin oxidizes faster to the ferric state than the adult hemoglobin and cytochrome b5 reductase activity is lower in the first few months of life (normally 60% of the normal adult level).

Congenital methemoglobinemia due to cytochrome b5 reductase activity deficiency can be type 1, cyanosis being the only clinically abnormality, or type 2, when cyanosis is accompanied by severe mental retardation and neurological impairment.

M-hemoglobin variants arise from point mutations in the alpha, beta or gamma globin genes changing amino acids located in the heme pocket. The most directly implicated are the proximal and distal hystidine but mutations in other amino acids have been described. In these circumstances the heme iron remains stabilised in the ferric state, and is relatively resistant to reduction by NADH-metHb reductase. In neonates the cyanosis associated with M-hemoglobin is more evident when mutations occur in alpha or gamma chains. In the latter, the methemoglobinemia is transient due to the changeover from fetal hemoglobin (Hb F $\alpha 2\gamma 2$) to the adult form (Hb A $\alpha 2\beta 2$).[1] Five Hb F-M due to G gamma chain variants have been described, all of them associated with transitory neonatal cyanosis (Table 1).[2-7]

The methemoglobinemias pattern of inheritance is autosomal recessive when caused by cytochrome b5 reductase deficiency and autosomal dominant when due to M-hemoglobin variants (reviewed by Percy MJ *et al*).[8]

Methemoglobinemia can also be caused by incidental exposure to environmental agents, such as aniline dyes, metoclopramide, chlorates and bromates. Clinically apparent methemoglobinemia occurs when levels are above 10%.

In acquired methemoglobinemias, the therapeutic approach is based on clinical severity, which correlates with metHb levels: when metHb ≥ 25% intravenous methylen blue or even hyperbaric oxygen chamber are necessary, when metHb <25%, and was caused by incidentally exposure to toxics, the treatment is cutaneous and/or gastric decontamination and oxygen therapy. In congenital methemoglobinemias, there is no specific treatment. It is important to avoid the administration of oxidative products (primaquine, sulphamides, xylocaine), and in some cases the administration of anti-oxidant products and activators of the alternative pathways of MetHb reduction (ascorbic acid, riboflavin and eventually methylene blue) may have some positive effect. Nevertheless, methylene blue is known to have little effect in Hemoglobin M diseases.[9]

Case report

A newborn baby presented with asymptomatic cyanosis less than one hour after delivery. He was the first son of a non consanguineous couple and was born at 38 weeks of gestation by cesarean section due to fetal placental insufficiency. Apgar score 9 at one minute and 10 at 5 minutes. Ten minutes after birth he developed central cyanosis with peripheral oxygen saturation of 77% and tachypnea, but without respiratory distress or neurological symptoms. He immediately started oxygen therapy with facial Mask with a FiO2 of 23%, with poor response. After admission at the neonatal Intensive care Unit (nICU), he was started on CPAP (continuous positive airway pressure) also without response. Liver and spleen were of normal

size. Cardiologic and respiratory evaluation did not reveal any malformation. He maintained central cyanosis with peripheral oxygen saturations between 80-85% with oxygen therapy 2l/min, without any other symptoms or respiratory distress for 5 days. On day 6 he became less cyanotic, his oxygen peripheral saturation was 88-90%, and he was discharged from the nICU to the pediatric ward.

Besides gestational diabetes, his mother had an unremarkable medical history without anemia, jaundice, gallstones or splenomegaly. His father was a premature baby requiring blood transfusion after birth, but was unaware of neonatal cyanosis. In adulthood he was asymptomatic with normal hematological parameters.

Newborn hematological and biochemistry studies were normal (Table 2), except for a metHb level of 16%.

The hemoglobins studies including hemoglobins scan were normal (Table 2).

Cytochrome b5 reductase activity was normal: 7,5 UI/gHb (newborn controls 6,6 – 9,8 UI/gHb).

As the clinical behaviour suggested a F-M Haemoglobin variant, even though hemoglobin studies were normal, we sequenced the gamma globin genes (HBG1 and HBG2), and a non previously described missense mutation at HBG2 gene, 85C-A (29 CTG-ATG), predictably changing leucine to methionine at amino acid 28 (Leu28Met), was identified in heterozygous state. The newborn was also found to be a carrier of HbF Sardinia, a frequent polymorphism of gamma globin chains.[10]

The HBG2 85C-A mutation was found in the father's genomic DNA, not associated with the HbF Sardinia polymorphism. This new Hb variant, named Hb F Viseu, after the patient's city of origin, has the same relative location as the adult Hb St Louis β28 (B10) Leu-Gln and Hb Chile β28 (B10) Leu-Met, described as unstable hemoglobin associated with chronic methemoglobinemia.[11,12]

Meanwhile, the baby showed a spontaneous and progressive remission of the cyanosis with a decline in metHb levels.

At the age of 8 weeks the patient showed no clinical evidence of cyanosis and metHb level was 1% at the fifth month as expected by the natural decreasing of G gamma chain synthesis.

Methods

Complete blood counts in whole blood EDTA samples were processed in an automated haematology analyser (Sysmex XE-2100).

Hemoglobin analysis of the hemolysate by HPLC (β Tal Short Program®, Variant II® Biorad) was used in the investigation of a structural variant.

Red blood cell cytochrome b5 reductase enzyme activity was performed spectrophotometrically, measuring the oxidation of NADH at a wavelength of 340 nm, according to methods standardized by ICSH.[13]

Measurement of metHb was performed by spectrophotometry at a wavelength of 630 nm following the addiction of cyanide and ferricyanide according to Dacie and Lewis.[14]

Sequence analysis of the globin genes HBG1, HBG2 were performed by dideoxy chain termination reaction using the Big-Dye ABI 310 sequencer *(Applied Biosystems, Foster City, USA)*.

Conclusion

Neonatal cyanosis associated with Hemoglobin F-M variants is a very rare condition. As far as reported in the literature, only five G gamma chain variants were identified – Hb-FM Osaka, Hb-FM Fort Ripley, Hb-FM Circleville, Hb F-Cincinnati and more recently Hb-FM Toms River (Table 1), all of them with single amino acid substitutions in residues crucial to normal heme-globin interaction.

Hb F Viseu, like the adult Hb St. Louis and Hb Chile, results from a leucine substitution at residue 28, located in the heme pocket near the H63 residue (His distal) (Figure 2. A)[15].

It was not possible to examine the stability of this new variant, but Hb F Viseu, like the adult Hbs St. Louis and Hb Chile, should be unstable, once the introduction in the heme pocket of an amino acid less hydrophobic and bigger than the wild-type residue (Figure 2. B, C) will confer instability to the molecule.

The wild type mutation have a structural and supportive function, the mutation might change the correct position of the H63 residue changing the oxygen-binding properties. As it was postulated to occur in Hb Chile, the methemoglobinemia observed in Hb F Viseu can be due to the intercalation of a molecule of water between the mutated γ28 amino acid and the distal histidine, γ63His, causing the heme iron to become permanently in the ferric state. [12] Hb St. Louis, although lacking the characteristic electrophoretic and spectral proprieties of M Hemoglobins, was considered one upon demonstration that its iron atoms are permanently in the ferric state.[16]

When evaluating a newborn with methemoglobinemia is important to bear in mind that it can be associated with an M-hemoglobin. Some of these Hb variants are electrophoretically silent and the diagnosis has to be done by globin chain genes sequencing.

Different hemoglobin variants have different forms of presentation. Even if the hemoglobin's studies are normal molecular characterization of globin genes can help to elucidate the diagnosis and avoid unnecessary complementary tests.

As postulated recently by Ostrander RJ, Green NS: "*An awareness of these conditions,* (inherited hemoglobinopathies) *among physicians can spare newborns and their families from unnecessary evaluation and worry*." [17]

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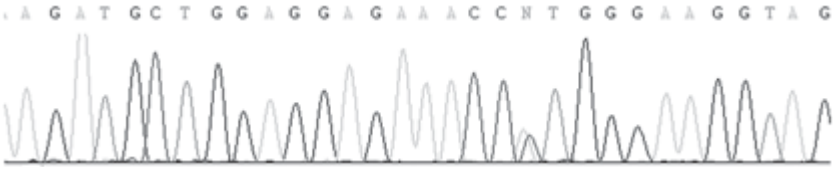

Figure 1. Forward nucleotide sequence of the γ^G globin gene.

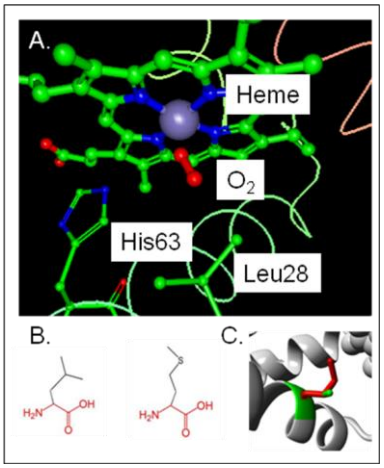

Figure 2. A. Ribbon representation of the heme pocket of the gamma chain, with the heme group, oxygen molecule, the distal His63 and the neighboring amino acid Leu28 B. Schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black. C. The protein is colored grey, the side chains of both the wild-type and the mutant residue are shown and colored green and red respectively.